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Isolation and Characterization of Chitosan from Local Mussels' Shell (*Pilsbryconcha* sp.)

Faiza A. Dali Teknologi Hasil Perikanan, Universitas Negeri Gorontalo, Indonesia Jl. Jend. Sudirman No. 6 Kota Gorontalo Email: dali.faiza@yahoo.co.id ABSTRACT This research aimed to utilize local mussels' shells in order to have economic value and to minimize the environmental pollutant through isolating and characterizing the chitosan of local shells (*Pilsbryconcha* sp.) from Gorontalo.

This method was conducted through preparing the sample, deproteinization process, demineralization, depigmentation, deacetylation and characterization of chitosan chitin. The isolation stage of 390 g of local mussels' shells produced 70.78 g of chitosan. The characteristics of chitosan from this process were white, powdery with 1% of moisture, ash content 0.09%, deacetylation degree 99%, soluble in CH<sub>3</sub>COOH 2%, insoluble in water, slightly soluble in condensed HCl and HNO<sub>3</sub>, insoluble in condensed NH<sub>3</sub> and insoluble in Na<sub>2</sub>SO<sub>4</sub> 2%. Keywords: Isolation, characterization, chitosan, shell, local mussels (*Pilsbryconcha* sp.)

INTRODUCTION Indonesian water has various types of mussels, one of them is in Lake Teratai, Boalemo, Gorontalo province. This local mussel (*Pilsbryconcha* sp.) is categorized as mollusk, with bilateral symmetrical shape that consists of two shells. This local mussel has not been optimally utilized. The utilization process currently limited to its meat, meanwhile the shells are thrown away as waste.

The waste from these mussels has become environmental problem, therefore, it is expected that the waste can be recycled to become something useful. Various research on shells of the arthropods, annelids, mollusks, coelenterates, and nematodes for their potentials to produce the chitin (Lesbani et. al, 2011). Chitin is a biopolymer with varied characteristics or chemical composition based on the source and the isolation process.

More than 80% distilled chitin is called chitosan. Chitin and chitosan have high economic values. Chitosan is widely used in food inashry, medical application, cosmetics, water processing, detergent, paper, textile, antimicrobial agent, antioxidant, edible film

inashry, and other biotechnology application (Ahmed et al 2010; Lee et al 2010; Bourbon et al 2011; Ahmed et al 2014; Trung and Bao 2015).

Chitin isolation can be done through demineralization process, deproteination, and depigmentation, meanwhile chitin transformation into chitosan can be conducted through deacetylation (No et al, 1989; Sofia et al 2010; Sarwar et al 2014). Characteristics of the chitosan product highly depend on the source of the animal and its production method.

This research aims to utilize the local mussels' shells into chitosan hence it has economic values and it would no longer be environmental pollutant, isolating and finding out the characteristics of chitosan of the local mussels' shells (*Pilsbryoconcha* sp.) from Gorontalo waters. **METHOD** The tools used in this research are oven, grinder, 100 mesh sieve, mixer, biker, thermometer, pH meter, analytical balance, sieve, heater, FTIR (Fourier Transform Infrared).

The ingredients used in this research are the shells of the local mussels obtained from the lake Teratai at Pontolo village of Boalemo district, Gorontalo), acetate acid (Merck), NaOH (Merck), HCl (Merck), HNO<sub>3</sub>, NH<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, aquades, H<sub>2</sub>O<sub>2</sub> 2%. **Sample Preparation** The mussels' shells taken from the lake Teratai of Pontolo village, Boalemo, Gorontalo washed with water (scrubbed when needed in order to get rid of the remaining dirt. Further, the shells were dried in an oven with 60 °C temperature for 6 hours.

When the shells were dried, they were grinded and sieved to produce shells' powder with the diameter of 0.25mm. This product is then used for extraction process. **Deproteinization** Sieved sample of the local mussels' shell were taken 390 g then put into the beaker with stirrer and thermometer. Next, 3.5% (w/v) of NaOH with 1:10 ratio was put into the beaker. Then, the mixture was heated in 90 °C temperature while stirred for 4 hours.

A sieving process followed this stage. The product of this process then washed using aquades up to a neutral pH was reached. The residue from this process then dried in an oven with 60 °C temperature for 24 hours. **Demineralization** The result from deprotenization process was put into a beaker and added with HCl 1 N solution with the ratio of 1:7.

Further, a room temperature heating was done while stirring it for 1 hour. The mixture then is sieved, the sediment then is washed using aquades. The residue (chitin) produced from this process then dried in an oven with 60 °C temperature for 24 hours.

Depigmentation The mineral-free residue (chitin) was then added with the 2% of H<sub>2</sub>O<sub>2</sub> solution with the ratio of 1:10. This mixture then sieved.

The sediment then washed with aquades to produce a neutral pH. The residue from this process then dried in an oven with 60 °C temperature for 24 hours. Deacetylation The residue powder was put into beaker with stirrer and thermometer, then 50% NaOH solution with 1:10 ratio was added into the beaker and stirred for 1 hour in 120 °C temperature, then, sieved. The residue from this process then washed with aquades until a neutral pH was obtained.

The product of this process then heated in an oven with 60 °C temperature for 24 hours. These deproteinization, demineralization, depigmentation, and deacetylation process referred to the result of Lukum's (2010) research. Characterization of chitin-chitosan Characterization used to differentiate chitin and chitosan stoichiometrically are the moisture level, ash level, and deacetylation degree using the spectrophotometric infrared. Moisture level testing (AOAC 2007), ash level testing (Sudarmadji et al 1994; AOAC 2007), chitosan solubility test.

Several solvents used in chitosan solubility test are 2% of CH<sub>3</sub>COOH, water, HCl, HNO<sub>3</sub>, NH<sub>3</sub>, and 2% Na<sub>2</sub>SO<sub>4</sub> (modified of Lukum, 2010). Further, deacetylation degree was determined using the FTIR method (Puspawati and Simpen, 2010). RESULTS AND DISCUSSION Isolation and characterization process of chitosan from local mussels' shells (*Pilsbryconcha* sp.)

from Gorontalo water was conducted several steps of deproteinization, demineralization, depigmentation, deacetylation, followed by moisture level test, ash level test, and determination of deacetylation degree and solubility level (by means of 2% of CH<sub>3</sub>COOH, water, condensed HCl, condensed HNO<sub>3</sub>, condensed NH<sub>3</sub>, and 2% of Na<sub>2</sub>SO<sub>4</sub>). Isolation process of 390 g of samples of local mussels' shells produced 70.78 g of white colored chitosan.

The deproteinization process and demineralization process were conducted to lower the yield produced, and after the depigmentation process a whiter color was obtained. Deproteinization is aimed to eliminate the protein in the sample by means of sodium hydroxide. Efficacy of protein elimination from chitin is influenced by alkaline level and temperature used.

According to Martati et al (2002) that the level of nitrogen residue in chitin significantly lowered when the temperature and deproteinization time are increased. Demineralization process is intended to eliminate the minerals content in the chitin such

as, CaCO<sub>3</sub> and CaPO<sub>4</sub>. On the other hand, depigmentation by using the H<sub>2</sub>O<sub>2</sub> is intended to get rid of any color substance using the solvent.

The solvents used in this process are mixture of ethanol-ether, sodium hypochlorite, chloroform, H<sub>2</sub>O<sub>2</sub>, ethyl acetate, and acetone. Deacetylation is a process of chitosan formulation from chitin by using NaOH to substitute the acetamide structure with amine structure. The elimination of acetyl structure using the 50% NaOH will influenced the level of deacetylation process of chitosan.

The success rate of chitin-chitosan deacetylation process is influenced by various factors, such as, NaOH concentrate, temperature, time, stirring in deacetylation process and the origin of sample. Hargono et al (2008) said that deacetylation process using the 50% of NaOH yields best quality chitosan. Within the solution, NaOH decomposed into Na<sup>+</sup> and OH<sup>-</sup>. Hydroxyl ion invades the electropositive carbon carbonyl.

The final products are chitosan and sodium acetate (shown in Figure). In Each steps from deproteinization to deacetylation, the residue were washed using the aquades in order to neutralize its pH. Figure. Chitin transformation to Chitosan The characterization result of chitosan from local mussels' (Pilsbryoconcha sp.) shells can be seen in the following Table. Table.

Chitosan characterization of local mussels' shell (Pilsbryoconcha sp.) from Gorontalo water

Parameter	Chitosan sample	Chitosan standard	Particle size	Powder	-Bubuk
Moisture level (% w/w)	1	< 10	Ash level (% w/w)	0,09	< 2
Deacetylation degree (%)	99	> 70	Solubility	2% CH <sub>3</sub> COOH	Water Condensed HCl Condensed HNO <sub>3</sub>
Condensed NH <sub>3</sub> 2%	Na <sub>2</sub> SO <sub>4</sub>	Soluble	Insoluble	Slightly soluble	Slightly soluble
Insoluble	Insoluble	Soluble	Insoluble	Slightly soluble	Slightly soluble
Insoluble	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Moisture level	Moisture level	Moisture level is one of the important parameter within the standard of qualified chitosan and will influence the storage time of the chitosan itself.			

The analysis result showed a relatively low moisture level (1%). Standard quality of chitosan dictates that the moisture level is no more than 10%, due to the higher the moisture level, the faster the damage process happened to the product. According to Sofia et al (2010) commercial chitosan produced by Sigma has 3.5% level of moisture.

The usage of NaOH in deproteinization process caused the softening of the cell's wall that will increase permeability of the cell's wall, hence, it enables moisture to be released from the cell's wall and help the drying process. Appropriate packaging and storage will maintain the low moisture level of chitosan (Martati et al, 2002). Ash Level The ash level in this research was lower (0.09%) than the ash level in chitosan from the shells of

Javanese crabs (8.01%) (Saputro et al, 2011), and has met the standard of chitosan.

Setting up the ash level aims to find out the extent of minerals content that have not been eliminated during the demineralization process. The usage of HCl was effective in eliminating inorganic minerals like  $\text{CaCO}_3$  and  $\text{CaPO}_4$ . No and Meyers (1995) said that the standard requirement for high quality chitosan has to have less than 1% of ash level.

According to Martati et al (2002) numerous dirt and residual mineral causing the ash level to be low is due to the temperature and length of deproteinization time.

Deacetylation degree Deacetylation degree of chitosan from the samples of local mussels' (*Pilsbryconcha* sp.) shell was 99%. Deacetylation degree in this research is higher than deacetylation degree (DD) of shrimp waste in Camacho et al (2010).

The high level of deacetylation degree shows that the chitosan produced from this research are pure chitosan. Deacetylation degree is one of the quality parameter of chitosan product that shows the percentage of acetyl structure that can be rid of both from the chitin and chitosan.

The higher the deacetylation degree of a chitosan, the lower the acetyl structure of that chitosan, hence, the interaction among ion and hydrogen chain will be stronger. Deacetylation degree of chitosan is influenced by the concentration of sodium hydroxide (NaOH) and the temperature during the processing. The bigger the concentration of NaOH solution, the higher the value of deacetylation degree (Apriani et al 2012; Hossain and Iqbal, 2014).

Solubility test The parameter that can also be used as standard in measuring the quality of chitosan is the solubility of that chitosan. The result of solubility test on 2%  $\text{CH}_3\text{COOH}$ , water, condensed HCl, condensed  $\text{HNO}_3$ , condensed  $\text{NH}_3$ , 2%  $\text{Na}_2\text{SO}_4$  are matched with the chitosan standard. Chitosan is soluble in acetate acid solution (2%  $\text{CH}_3\text{COOH}$ ), insoluble in water, condensed  $\text{NH}_3$  and 2%  $\text{Na}_2\text{SO}_4$ , slightly soluble in HCl and condensed  $\text{HNO}_3$ .

Chitosan solubility in  $\text{CH}_3\text{COOH}$  is due to the high temperature during the deacetylation process. Savitri et al (2010) stated that the higher the concentration of NaOH and the temperature during deacetylation process, the higher the solubility of chitosan within the  $\text{CH}_3\text{COOH}$  and the more the acetyl structure being replaced, hence the value of deacetylation degree increased.

Chitosan is insoluble in water, strong alkaline solution, and slightly soluble in HCl and  $\text{HNO}_3$ , and soluble in acetate acid and formiate (Apriani et al 2012). CONCLUSION

Based on the findings of this research on the **isolation and characterization of chitosan from local mussels'** shells (*Pilsbryconcha* sp.) of the Gorontalo lake it is concluded that the local mussels' shells can be utilized as chitosan. 70.78 grams of white colored powder chitosan was produced from deproteinization stage, demineralization, depigmentation, and deacetylation stage.

This chitosan has 1% of moisture level, ash level of 0.09%, deacetylation degree 99%, soluble in 2% of CH<sub>3</sub>COOH, insoluble in water, condensed NH<sub>3</sub> and 2% of Na<sub>2</sub>SO<sub>4</sub>, slightly soluble in condensed HCl and condensed HNO<sub>3</sub>.

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